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Unifying theory of hypoxia tolerance: Molecular/metabolic defense and rescue mechanisms for surviving oxygen lack

(oxygen sensing/hypoxia defense/turtle hepatocytes/turtle brain)

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ABSTRACT **We develop a unifying theory of hypoxia tolerance based on information from two cell level models (brain cortical cells and isolated hepatocytes) from the highly anoxia tolerant aquatic turtle and from other more hypoxia sensitive systems. We propose that the response of hypoxia tolerant systems to oxygen lack occurs in two phases (defense and rescue). The first lines of defense against hypoxia include a balanced suppression of ATP-demand and ATP-supply pathways; this regulation stabilizes (adenylates) at new steady-state levels even while ATP turnover rates greatly decline. The ATP demands of ion pumping are down-regulated by generalized "channel" arrest in hepatocytes and by "spike" arrest in neurons. Hypoxic ATP demands of protein synthesis are down-regulated probably by translational arrest. In hypoxia sensitive cells this translational arrest seems irreversible, but hypoxia-tolerant systems activate "rescue" mechanisms if the period of oxygen lack is extended by preferentially regulating the expression of several proteins. In these cells, a cascade of processes underpinning hypoxia rescue and defense begins with an oxygen sensor (a heme protein) and a signal-transduction pathway, which leads to significant gene-based metabolic reprogramming—the rescue process—with maintained down-regulation of energy-demand and energy-supply pathways in metabolism throughout the hypoxic period. This recent work begins to clarify how normoxic maintenance ATP turnover rates can be drastically (10-fold) down-regulated to a new hypometabolic steady state, which is prerequisite for surviving prolonged hypoxia or anoxia. The implications of these developments are extensive in biology and medicine.**

Cell Level Model of Hypoxia Tolerance

Biologists have long known that certain vertebrate species have evolved capabilities for surviving prolonged periods with limited supplies of oxygen. Studies of such species [some so anoxia tolerant that they are referred to as "facultative" anaerobes (1)] have revealed several widely used strategies of hypoxia adaptation. Two of the most significant of these are: (*i*) severe down-regulation of energy turnover (2–7) and (*ii*) up-regulation of energetic efficiency of ATP-producing pathways (8). The latter involve stoichiometric efficiencies. In hypoxia adaptation, pathways that maximize the yield of ATP per mol of O_2 are favored, whereas in anoxia adaptation, anaerobic pathways are favored that maximize the yield of ATP per mol of H^+ formed in the fermentation (9, 10). In hypoxia adaptation, the ratio of anaerobic/aerobic metabolic potentials may be up-regulated, coincident with up-regulation of [glycogen] (fermentable substrate) and of buffering capacities (2). Although all these mechanisms may be useful in surviving hypoxia, evaluation of such defense strategies naturally evolved by hypoxia-tolerant animals shows that suppression of energy turnover supplies the greatest protection against, and hence, advantage in, hypoxia. The immense advantage of this defense strategy is widely appreciated by many biologists (4, 5, 11–13). In one of his last personal communications to one of the authors (P.W.H.), the great comparative physiologist Kjell Johansen referred to this strategy as "turning down to the pilot light" and he, like many earlier workers, was acutely aware of its relative importance. Although recognized as a kind of hallmark of reversible entry into and return from states of severe O_2 deprivation, a number of unexplained problems have remained. In particular, it has not been clear (*i*) how cells/tissues "know" when to turn on their hypoxia defense mechanisms, (*ii*) which pathways of ATP demand and ATP supply are down-regulated or by how much, (*iii*) how membrane electrochemical gradients are stabilized, and (*iv*) what gene-expression and protein-expression level adjustments are involved in hypoxic reorganization of cell structure and function. Recent studies of a well-known vertebrate "facultative anaerobe," the aquatic turtle, used brain cortical slices to probe electrophysiological properties of neurons under anoxia (16–19) and isolated liver hepatocytes to probe cell level biochemical responses to anoxia (20–24). When integrated with independent lines of research in other laboratories, these studies supply the raw material for developing a synthetic cell-level model or general hypothesis of hypoxia tolerance, which goes a long way to answering the above unresolved questions.

How Cells Detect When Oxygen Becomes Limiting

Traditionally, biologists' views of cell level responses to O_2 limitation are formalized in the concept of the Pasteur effect; as ATP generation by oxidative phosphorylation begins to fall off due to O_2 lack, the energetic deficit is made up by activation of anaerobic ATP supply pathways. In the case of turtle liver cells, this kind of O_2 sensing would mean activation of anaerobic glycolysis (since this is the only known mechanism for ATP generation without O_2 in these cells). Two observations argue persuasively against this as the means for sensing hypoxia in tolerant cells. First, hypoxia-tolerant cells/tissues use anaerobic metabolism not to make up energy deficits but to sustain a reduced energy turnover state instead (3, 4, 11, 14, 15). Second, responses to falling [O_2] begin at concentrations (3) much higher than the Km(O_2) for mitochondria (termed oxygen conformity). In fact, it was the observation of oxygen conformity that first led workers in this area to postulate mechanisms more widespread than the erythropoietin (EPO)

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Abbreviations: EPO, erythropoietin; EF1α, elongation factor 1α; PGK, phosphoglycerate kinase; LDH, lactate dehydrogenase; HIF1, hypoxia-inducible factor 1.

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system (25) for sensing O_2 as the cell level means for detecting hypoxia (10, 11, 13, 26) (see below).

Balancing ATP-Demand and ATP-Supply Pathways During Hypoxia

Based on the observation of oxygen conformity and on earlier whole-organism studies, it was not surprising to find that, when liver hepatocytes encounter O_2 lack, they suppress energy turnover by a factor of almost 10-fold (20, 21). All else being equal, 1 mol of ATP could sustain these cells for 10 times longer than under normal conditions. As mentioned, this strategy is frequently observed in hypoxia-tolerant cells, but to put the strategy into perspective, it is important to know quantitatively which processes are turned down. Thus, we assessed the main energy demand functions under normoxia (energetically balanced by the O_2 consumption rates under these conditions) and compared these to the energy sinks remaining under anoxia. We found that under normoxic conditions, the main energy sinks (Table 1) are (*i*) protein synthesis, (*ii*) protein degradation, (*iii*) Na^+/K^+ pumping, (*iv*) urea biosynthesis, and (*v*) glucose biosynthesis. For practical purposes, the ATP demands of these processes account for essentially 100% of the ATP production expected from O_2 consumption (20–24).

Most instructive is what happens to the same processes in turtle liver cells under anoxia. Under these conditions, the ATP demand of protein turnover drops to less than 10% of normoxic rates; urea biosynthesis drops to essentially zero, as does the biosynthesis of glucose (not unexpectedly, because a major role of the liver under anoxic conditions is to supply glucose for the rest of the body). Although the ATP demands of the Na^+/K^+ ATPase are also drastically reduced, the suppression in percentage terms is less than for overall ATP turnover. As a result, under anoxic conditions, the Na^+ pump becomes the dominant energy sink of the cell, accounting for up to 75% of the ATP demand of the cell (20). Although there may well be other minor energy demand processes remaining under anoxic conditions, the ATP demand pathways identified during anoxia fully account for the ATP generated by anaerobic glycolysis (Table 1).

Integrating Metabolism and Membrane Functions During Hypoxia

Even if, in percentage terms, ion pumping (as assessed by the activity of Na^+/K^+ ATPase) is the single largest ATP sink during anoxia in turtle hepatocytes, its absolute ATPase or pumping activity is only a small fraction (about one-fourth) of normoxic levels. However, direct estimates indicate that the electrochemical potential in anoxic liver cells is essentially the same as in normoxia (20). The only mechanism by which we can account for (*i*) the large scale drop in absolute Na^+/K^+ ATPase activity and (*ii*) the simultaneous maintenance of normal electrochemical gradients is by means of a similar magnitude decrease in cell membrane permeability [termed generalized "channel arrest" in the literature (11)].

Table 1. The main ATP-demand pathways during normoxia and anoxia in turtle hepatocytes

Pathway	ATP demand, μmol ATP $\times$ g^{-1} $\times$ h^{-1}		
	Normoxia	Anoxia	% suppression
Total	67.0	6.3	94
Na^+ pump	19.1	4.8	75
Protein synthesis	24.4	1.6	93
Protein breakdown	11.1	0.7	94
Urea synthesis	2.0	0.6	70
Gluconeogenesis	11.4	0.0	100

Modified from Buck and Hochachka (20) and Land and Hochachka (22, 23).

In our first attempt at a synthesis in this research (11), a channel arrest component of a hypoxia-tolerance theory postulated (*i*) that hypoxia-tolerant cells would have an inherent low permeability (either low-channel densities or low-channel activities) and (*ii*) that they would sustain a further suppression of membrane permeability to ions when exposed to oxygen lack (further channel arrest by either suppression of channel densities or channel activities). Turtle liver cells display both of these characteristics (especially when compared with mammalian homologs); thus, they clearly fit the classical definition of metabolic and channel arrest as two telling signatures of hypoxia tolerance [observed also in hypoxia-tolerant fish hepatocytes (14, 15)].

In contrast, in turtle cortical cells, only the first criterion is met: a background electrical conductivity that is unusually low and when compared at biological temperatures (15°C vs. 37°C) can be as low as 1/25th the conductivity of cell membranes of rat cortical cells (18). However, when exposed to acute O_2 lack (for up to several hours), there is no further channel arrest and, therefore, no further decrease in background conductivity of these neuronal cells. We consider that to be an important reason why the main energy saving mechanism in turtle brain (27, 28) is down-regulation of firing rates or synaptic transmission [termed 'spike arrest' by Sick *et al.* (28)], presumably through adenosine-mediated down-regulation of excitatory amino acid (especially glutamate) release with concomittant increase in release of inhibitory amino acids (27, 29). This may also explain why the metabolic suppression in brain is less than in liver cells, down to about one-half rather than 1/10th of normoxic rates (19). A related implication is that the ATP turnover rates of anoxic turtle neurons are higher than in turtle liver cells [consistent with microcalorimetry (19, 21)].

Hypoxic Suppression of Protein Synthesis

The only energy requiring process in normoxic liver cells that is more costly than ion pumping is protein turnover (20–24), so evaluating this ATP sink under oxygen limiting conditions is of particular importance. Interestingly, one of the first effects of hypoxia on cell metabolic functions is a rapid, large magnitude inhibition of protein synthesis. The decline can be so rapid that its time course is difficult to ascertain accurately with currently used techniques for measuring protein biosynthesis (30).

In principle, a hypoxia-induced block could occur at the level of gene transcription or at translation. In animal (31, 32) and in plant systems (33), hypoxic suppression of protein synthesis may be mediated by a translational block affecting both initiation and elongation. Inhibition of initiation is not well understood, but inhibition of elongation at least in plant systems appears to depend upon an accumulation of EF1α, which at low pH appears to form nonfunctional complexes with polysome-associated mRNA (33). Because the role of this elongation factor is to present amino acyl-tRNA to the A site of ribosomes, it is easy to visualize how failure to dissociate from polysomes would prevent peptidyl synthesis and translocation. In hypoxia-sensitive systems, such as rat hepatocytes (30), hypoxia-induced translational arrest and, thus, blockade of protein synthetic capacity seems to remain general for at least 2 hr (longer term experiments with this preparation are not realistic due to cell damage and cell death).

Preferential Gene Expression During Extended Hypoxia

Although "rescue" of protein synthesis in hypoxia-sensitive mammalian cells does not seem feasible without O_2, exactly such a rescue system seems available to hypoxia tolerant cells (24, 33). One underlying rescue mechanism appears to require

the overproduction of key elongation factors such as EF1α; with sustained hypoxia, the latter is overexpressed and accumulates throughout the stress period. Although the mechanism for selective translation (of only specific messages) is not well understood, this rescue mechanism is known to preferentially favor expression of glycolytic enzymes (33) whose activity must be sustained to survive O_2 lack.

When we turned our attention to this issue in turtle hepatocytes we found (23, 24) that the situation was more complex. Under conditions of prolonged O_2 lack, the expression of four proteins in turtle hepatocytes was preferentially up-regulated, whereas the expression of five proteins was preferentially down-regulated. The hypoxia dependence of this system appeared similar to O_2 sensing and regulatory pathways in EPO biosynthesis (34–40). The field of EPO research developed four experimental criteria for discriminating between O_2 sensing and signal transduction pathways vs. simple O_2-dependent metabolic pathways. First, in the former, the response should be negated by normoxia even in the presence of metabolic poisons such as cyanide—as observed in the turtle hepatocyte gene expression changes in anoxia. Second, in heme protein O_2-sensing pathways, Co^{2+} or Ni^{2+}, which lock heme proteins in their deoxy conformation, should mimic anoxia effects—as observed in turtle hepatocyte gene expression changes. Third, in heme protein O_2-sensing pathways, carbon monoxide, which locks heme proteins in oxy conformation, should reverse anoxia effects—also observed in the turtle hepatocyte studies. Finally, heme synthesis inhibitors should abrogate the effects of Co^{2+} or Ni^{2+} in heme protein based O_2-sensing pathways—again observed in the turtle hepatocyte protein expression changes in anoxia (23, 24).

These studies, inspired by developments in EPO research, led to the conclusion that a putative heme protein based O_2-sensing and signal transduction system in turtle hepatocytes serves to activate the expression of a group of several proteins during extended periods of O_2 limitation, as well as to simultaneously further slow down the expression of another group of several proteins. The O_2-sensing pathway in turtle hepatocytes is probably homologous to that found in rat liver cells; in the latter (41, 42), hypoxia sensing and signal transduction seems to serve in negatively modulating the effects of glucagon on gluconeogenic enzymes such as phospho*enol*pyruvate carboxykinase. Similar O_2-sensing and signal transduction control systems may be basic to the hypoxic up-regulation of genes for glycolytic enzymes (43), and to the hypoxic suppression of Krebs cycle enzymes (44).

So far, perhaps the most extensive analysis of the regulation of a putative homologous O_2-sensing system derives from studies of EPO regulation (34). In evaluating their postulate of universal O_2-sensing and response pathways, Firth *et al.* (35) recently confirmed that the genes for human phosphoglycerate kinase (PGK) 1 from human cancer cell lines and for mouse muscle type lactate dehydrogenase (LDH) A from mouse fibroblasts are induced by hypoxia; expression is favored by Co^{2+} but not cyanide and blocked by protein synthesis inhibition. The cis-acting control sequences, necessary for the hypoxic PGK induction, are located in the 5′ flanking region of the PGK gene and are seemingly homologous to the so-called hypoxia-inducible factor 1 (HIF1)-binding site within the EPO enhancer (3′ flanking region of the EPO gene), which regulates EPO gene function (38–40). Similar studies of the LDH A 5′ flanking (or promoter) region define three domains required for hypoxia-regulated LDH expression. The first of these is an HIF1-binding site and is an absolute requirement for hypoxia-sensitive function. Site two, 5′ to the HIF1 site, is similar to a critical cooperative site in the EPO enhancer, whereas site three (3′ to the HIF1 site) has the motif of a cAMP response element (36). Interactions between all three sites in the LDH A promoter are required for the maximum hypoxic inducibility, but the physiological significance of interactions between the HIF1 and cAMP response element sites, between oxygen-initiated and cAMP-initiated signal transduction pathways, remains to be clarified (36). Nevertheless, the hypoxia-sensitive expression of each of these three protein systems (EPO, PGK, and LDH) and of glycolytic enzymes in other systems (37, 38) appears to be a classical two-step or two-cycle system: the first cycle regulates the expression of a key hypoxia-sensitive transcription factor (HIF1), whereas the second cycle of gene expression is mediated by HIF1 and regulates the biosynthesis of EPO, PGK, LDH, and other glycolytic enzymes *per se* (37).

Since the expression of other proteins, such as elongation factors, may be similarly regulated, it is tempting to consider that the induction and accumulation of EF1α in hypoxic plant tissue and the hypoxia-sensitive proteins in turtle and rat hepatocytes are under similar two-cycle gene regulation (Fig. 1), although at this time such homology has not been demonstrated. Nevertheless, all the data considered together are consistent with the hypotheses (*i*) that the heme protein based O_2 sensor and the O_2-regulated control elements are widespread and possibly universal in eukaryotes; (*ii*) that initial hypoxia inducible proteins are regulatory factors, such as HIF1 (a transcription factor) and EF1α (an elongation factor); and (*iii*) that additional hypoxia inducible proteins in turtle liver cells are glycolytic enzymes such as PGK, pyruvate kinase, and LDH, which must be sustained during O_2 lack, whereas the suppressed loci may well include genes for enzymes such as phospho*enol*pyruvate carboxykinase involved in gluconeogenesis and for enzymes in mitochondrial O_2-dependent metabolism.

The above examples are not the only genes whose expression is hypoxia dependent. Other examples of hypoxic gene regulation include the c-*fos* and c-*jun* system (45–47), hemeoxy-

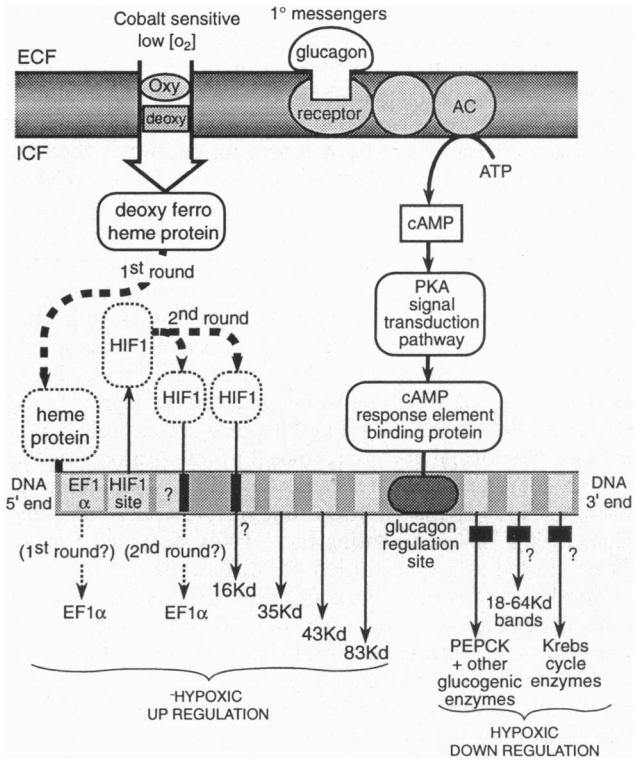

FIG. 1. Schematic diagram of O_2-sensing and signal transduction components thought to be involved in metabolic hypoxia defense response in turtle and rat hepatocytes. The diagram is based on the EPO model (see text). Hypothetical two rounds of gene regulation are connected with dotted arrows to indicate that in turtle hepatocytes the control circuitries are assumed to be homologous to the EPO system in EPO producing cells. Summary based on studies of turtle and rat hepatocytes (24, 41, 42).

genases (48), genes in hypoxia-activated apoptosis (49), and genes for stress proteins or chaperones (50). The hypoxia-sensitive regulation of the c-*fos* and c-*jun* protooncogenes in neonatal rat cardiac myocytes is probably more complex than any of the examples thus far considered (45, 46). (At least one rationale for using neonatal myocytes for these studies was to take advantage of a naturally evolved hypoxia or ischemia tolerance, the same rationale as was used in the above-mentioned work on turtle hepatocytes.) Both c-*fos* and c-*jun* are genes for transcription factors and represent two members of a family frequently referred to as classical "immediate early genes," one of the most rapidly inducible of genes in the cell; their general functional roles are to regulate growth, differentiation, and reprogramming or restructuring of cells in different physiological states. The different members of this gene family are induced to variable degree by various stimuli and can act in a synergistic or opposing manner, suggesting that both the absolute and the relative amounts of different members of the family determine their net effects (51–52). The c-fos and c-jun protein products are separately inactive; however, as heterodimers, they form a part of the AP1 transcription factor complex and are involved in regulating the expression of a battery of other genes with AP1-binding sites in their promoter regions. This regulatory cascade is considered to orchestrate "adaptive responses" to various stimuli including hypoxia, ischemia, pressure overload, stretch, and several hormones (45, 46), all of which, if sustained, are characterized by cellular reprogramming and metabolic reorganization (51–54). At least in response to hypoxia, the signal transduction pathway is protein kinase C dependent. Interestingly, because jun is a sulfhydryl protein, this tertiary messenger can be directly modulated by hypoxia (or by redox) and in this way can also influence the overall hypoxia-initiated flow of information from outside of the cell to the genes (Fig. 2).

The hypoxia sensing components of this immediate early response system in myocytes have not been as systematically dissected as has the EPO system. Yet it is clear, that if they are homologous to the EPO oxygen sensing pathways, then the c-*fos*/c-*jun* regulatory system would involve three cycles of gene activation. We interpret this (three cycle) system as constituting the final and perhaps most complex process level in the rescue phase of development of hypoxia tolerance; i.e., such AP1-mediated regulation of genes during hypoxia is considered basic to the remolding (23, 53, 54) and stabilization of the cell for sustained survival without oxygen. Interestingly, such roles—representing the epitomy of rescue of the cell from destructive cascades leading to hypoxic cell damage (11) and cell death (49)—during sustained hypoxia are entirely consistent with the normal normoxic roles for these protooncogenes in the regulation of genes for cell growth, development, and differentiation (51–52). In this frame of reference, stabilizing, restructuring, or consolidating the cell for sustained hypoxia can be viewed as a special kind of "differentiation."

Finally, the c-fos and c-jun transcription factors are involved in the up-regulation of genes for enzymes (such as superoxide dismutases and glutathione *S*-transferases) functioning in detoxification of end products derived from oxidative metabolism during recovery (55–60). A similar "anticipatory" protective role is the explanation given for increased expression of such enzymes during O_2 limitation in hypoxia tolerant reptiles (57, 58). It is also the accepted explanation for activation of hemeoxygenase gene expression signaled by hypoxia (48). Be that as it may, these exciting studies in several independent lines of investigation go a long way toward explaining how hypoxia tolerant cells "know" when conditions become hypoxic or anoxic and "know" which response pathways to use to turn on hypoxia defense and rescue processes *per se.*

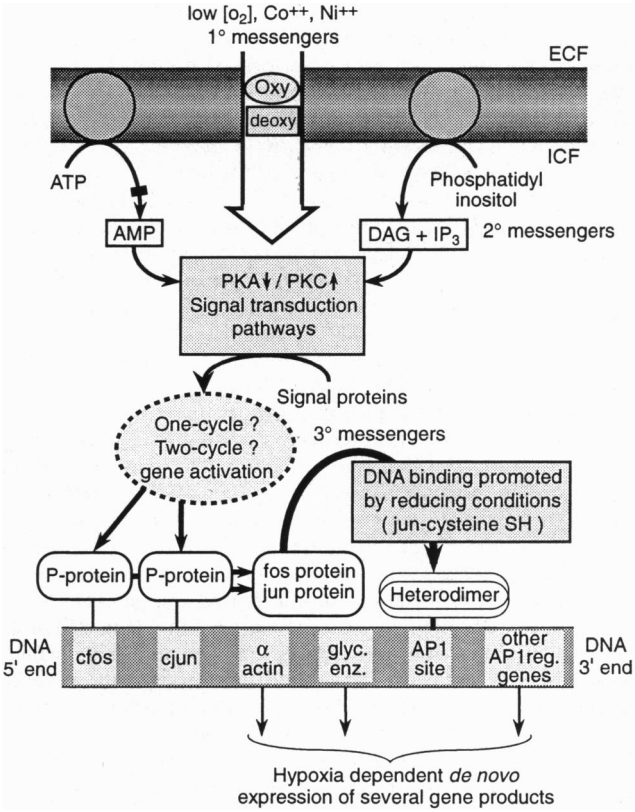

FIG. 2. Schematic diagram of O_2-sensing and signal transduction components thought to be involved in immediate–early gene expression responses to hypoxia in neonatal rat heart myocytes. The second messenger signal transduction pathways seem to involve inositol triphosphate (IP_3), diacylglycerol (DAG), and protein kinase C (PKC). The products of c-fos and c-jun (tertiary messengers) contribute to formation of AP1 (activator protein 1), a heteropolymer whose main role is to modulate expression of genes further downstream in the regulatory circuit [based on Webster *et al.* (45, 46) and references therein].

Stabilizing Adenylate Concentrations

An additional insight is that despite hypoxic reprogramming turtle liver cells and neurons, remain in energy balance. Because ATP demand and anaerobic supply pathways remain in balance, the concentrations of the high-energy phosphate metabolites are sustained in the normal range as [glycogen] declines and [lactate] and [H^+] rise (17–24). In other hypoxia-tolerant cells, [adenylates] may decline modestly (14, 15), or even drastically, as in some invertebrate cells (7), to a new steady state. This condition (7, 14, 15, 19, 21, 27, 28, 61, 62) contrasts with the non-steady-state situation in hypoxia-sensitive cells with continuously declining [ATP] during O_2 lack (15, 28). Even if the decline in [ATP] is not considered the cause of cell death during O_2 limitation in hypoxia-sensitive cells (28, 63), the maintenance of energy balance with stable [ATP] in hypoxia-tolerant cells is taken to be a signature of effective defense against hypoxia (27).

Conclusions

From these studies it is now possible to construct a working hypothesis for how hypoxia-tolerant cells respond to O_2 lack (Fig. 3). During the very early acute phases, a poorly understood hypoxia-sensing and signal transduction system orchestrates a series of molecular defense processes, which include

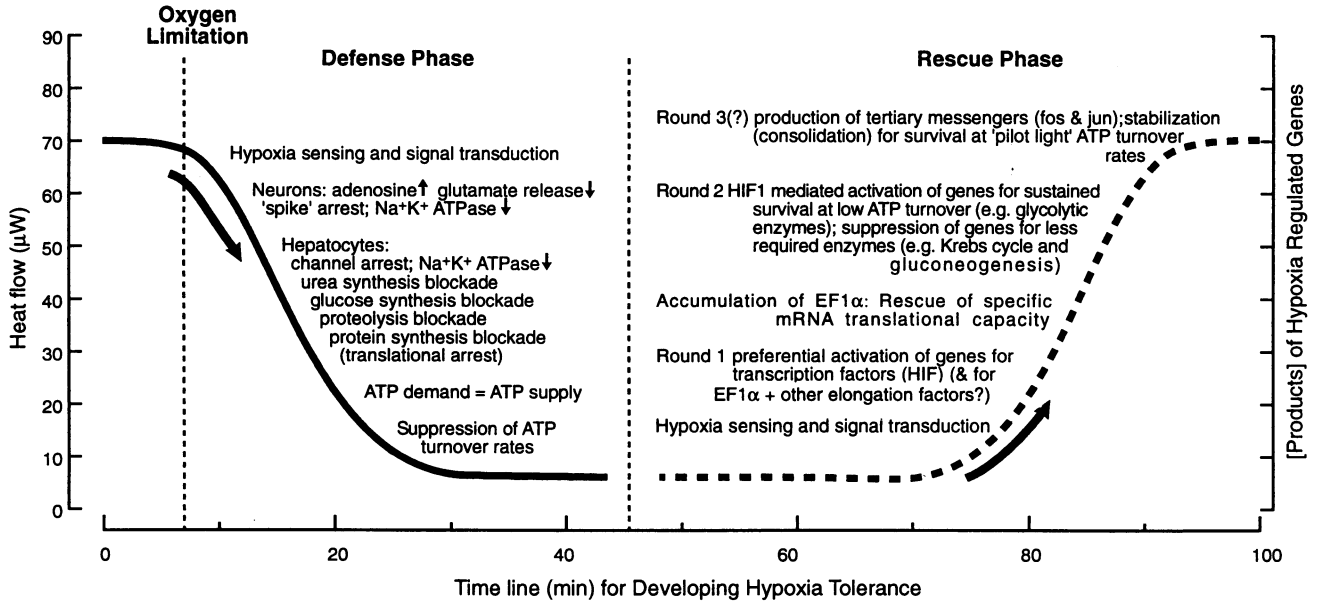

FIG. 3. Summary model of molecular defense (*Left*) and rescue (*Right*) processes thought to account for the extreme tolerance to oxygen limitation expressed by cells from hypoxia-tolerant species. (*Left*) Probable sequences are ordered from top to bottom (direction of arrow); vertical axis is heat output of turtle hepatocytes (21). (*Right*) Temporal order of rescue processes is not as well known; although some of these processes may occur simultaneously, a possible sequence of rescue events is arranged from bottom to top (indicated by direction of arrow). (*Right*) Vertical axis: hypoxia-induced products expressed as percent of maximum hypoxia-inducible response (see refs. 23 and 24) for magnitude in turtle hepatocytes. The timeline for hypoxia tolerance is based on turtle liver cells (21–24); for other systems, the time required for analogous or homologous processes may differ. Even within the same species, the timeline can vary for different tissues; for example, turtle cortical neurons require longer times to mount and complete their defense phase than do liver cells. EF1α accumulation allowing rescue of translation in hypoxia-tolerant plant cells may require several hours (33). Less time may be required for hypoxia induction in turtle hepatocytes (24, 25) than in more hypoxia-sensitive mammalian cells (35, 36).

(*i*) a 90% or greater global decline in protein biosynthesis, possibly due to low pH-mediated polysome–EF1α complexing (translational arrest), and

(*ii*) a generalized decline in membrane permeability ("channel arrest") or in firing frequence ("spike arrest") in the case of nervous tissue and, thus, in the ATP demand for ion pumping.

These two processes together account for most of the energy savings that allow the very low ATP turnover rates requisite for long-term hypoxia survival. These adjustments are only possible because of

(*iii*) a coordinated suppression of ATP supply pathways so as to maintain supply-demand balance and thus stable adenylate concentrations.

The acute or defense phase of hypoxia tolerance is considered to blend almost imperceptibly into a secondary series of processes; in the literature, these are variously termed immediate-early gene responses (45) or acclimatory expression adjustments (2, 3). As the combined effect of these processes is to reactivate some mRNA translational capacities and probably to consolidate and stabilize the cell at strikingly reduced ATP turnover rates (at the "pilot light"), we refer to these combined processes as a rescue phase for establishing hypoxia tolerance. The rescue phase includes (Fig. 3)

(*iv*) heme protein based, hypoxia sensing and signal transduction pathways that activate one-cycle gene expression for the production of key transcription factors (such as HIF1); one-cycle gene regulation may also allow continued production of key elongation factors and so seemingly rescue the cell translational capacities for specific mRNAs during continued O_2 lack,

(*v*) sets of two-cycle, hypoxia-sensitive genes (probably including genes for for glycolytic enzymes) whose expression is up-regulated during prolonged O_2 limitation (protein products of these genes are presumably involved in stabilizing cell operations at severely suppressed ATP turnover rates during hypoxia; the gene for EF1α could be regulated by such two-cycle, rather than one-cycle, gene-activation circuits),

(*vi*) sets of two-cycle, hypoxia-regulated genes (possibly including genes for enzymes in gluconeogenesis and the Krebs cycle), whose expression is down-regulated during prolonged O_2 limitation (protein products of these genes presumably are not needed, or are not as critical, for survival without O_2), and possibly

(*vii*) sets of more complex, two- or three-cycle hypoxia-regulated genes (such as c-*fos* and c-*jun*), whose products appear as tertiary messengers in the expression regulation of (probably numerous) other housekeeping genes (involved in restructuring, consolidation, and stabilization of cell functions at the severely suppressed ATP turnover rates requisite for surviving prolonged hypoxia). These latter, more complex regulatory phenomena make it easier to understand features of the O_2-limited cell, such as suppressed proteolysis, increased protein stability, and induction of chaperone proteins, features indicative of extensive reprogramming of the normoxic cell to generate the hypoxia tolerant cell.

Empirically, we know that the combination of these molecular processes allows cells and tissues of hypoxia tolerant species to greatly extend the length of time they are able to survive under hypoxic or even anoxic conditions. But it is clear that summaries like those in Fig. 3 only supply key highlights and key processes of hypoxia tolerance. Further work is required for filling in the details of such generalized maps of the nature of hypoxia tolerance—and for evaluating the molecular defense mechanisms required during recovery and reperfusion (a serious problem area only mentioned in passing in this analysis). Equally daunting is the challenge of discovering whether or not these strategic mechanisms can be transferred from hypoxia-tolerant to hypoxia-sensitive cells—which is the Holy Grail for many hypoxia researchers in the medical field.

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